

Europäisches Patentamt
European Patent Office
Office européen des brevets



AP

(11) **EP 1 361 222 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
12.11.2003 Bulletin 2003/46

(51) Int Cl.7: **C07D 305/14, A61K 31/337,
A61P 35/00**

(21) Application number: **03008433.9**

(22) Date of filing: **14.03.1994**

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE**

(30) Priority: **19.03.1993 US 33598**

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
94301809.3 / 0 617 018

(71) Applicant: **BRISTOL-MYERS SQUIBB COMPANY**
Princeton, NJ 08543-4000 (US)

(72) Inventors:
• **Thottathil, John K.**
Robbinsville, NJ (US)

- **Trifunovich, Ivan D.**
Pittstown, NJ 08867-4238 (US)
- **Kucera, David J.**
Del Mar, CA 92014-2960 (US)
- **Li, Wen-Sen**
Holmdel, NJ 07733-1274 (US)

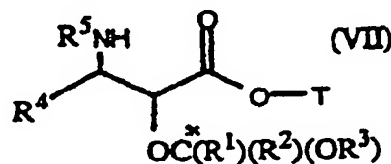
(74) Representative: **Clyde-Watson, Zöe et al**
D Young & Co
21 New Fetter Lane
London EC4A 1DA (GB)

Remarks:

This application was filed on 11 - 04 - 2003 as a
divisional application to the application mentioned
under INID code 62.

(54) **Sidechain-bearing taxanes**

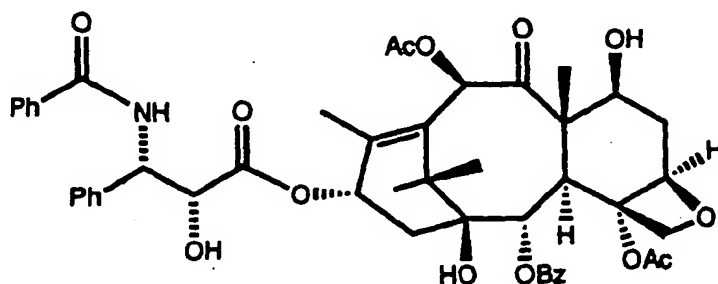
(57) Novel β -lactams find utility as intermediates in the preparation of sidechain-bearing taxanes such as taxol and taxol derivatives. The present invention relates to novel sidechain-bearing taxanes of formula (VII).



Description

[0001] The present invention relates to novel β -lactams. The β -lactams of the present invention find utility as intermediates in the preparation of sidechain-bearing taxanes such as taxol and taxol derivatives. The present invention also relates to novel methods of coupling β -lactams to form such sidechain-bearing taxanes, and to novel sidechain-bearing taxanes.

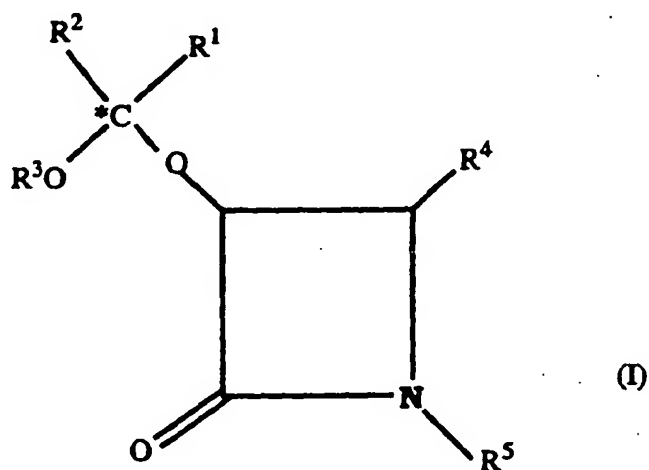
[0002] Taxanes are diterpene compounds having utility in the pharmaceutical field. For example, taxol, a taxane having the structure:



where Ph is phenyl, Ac is acetyl and Bz is benzoyl, has been found to be an effective anticancer agent.

[0003] Naturally occurring taxanes such as taxol may be found in plant materials, and have been isolated therefrom. Such taxanes may, however, be present in plant materials in relatively small amounts so that, in the case of taxol, for example, large numbers of the slow-growing yew trees forming a source for the compound may be required. The art has thus continued to search for synthetic, including semi-synthetic routes for the preparation of naturally occurring taxanes such as taxol, as well as routes for the preparation of synthetic, pharmaceutically useful analogs thereof.

[0004] The present invention provides novel β -lactam compounds of the following formula I:



where

R¹ and R² are:

- (i) both the same alkyl group;
- (ii) together form a cycloalkyl group;
- (iii) together form a cycloalkenyl group; or
- (iv) together form a heterocyclo group;

R³ is alkyl;

R⁴ is aryl;

R⁵ is hydrogen, arylcarbonyl, or alkyloxycarbonyl, and salts thereof.

[0005] The β -lactams of the present invention are useful as intermediates in the preparation of sidechain-bearing taxanes such as taxol and taxol derivatives. In particular, these compounds may be coupled with a taxane moiety to form the aforementioned sidechain.

[0006] As the stereochemistry of taxanes may affect their pharmaceutical activity, it is desirable to employ β -lactam intermediates which will provide the final taxane product with the stereochemistry sought. In the β -lactams of the present invention, the carbon marked with an asterisk in the above formula I is an asymmetric carbon. Where such a carbon center is asymmetric, a mixture of diastereomers can be formed. The β -lactams of the present invention provide superior results relative to β -lactams which contain an asymmetric carbon at the corresponding position since, when the latter compounds are prepared, or when they are coupled with a taxane moiety, products are formed as a mixture of stereoisomers. The formation of such a mixture of stereoisomers results in an inefficient use of the starting materials, and complicates separation and purification procedures.

[0007] The β -lactams of the formula I of the present invention are further advantageous in terms of the yield and purity of the final taxane product. In particular, the β -lactams of the present invention allow efficient conversion, and therefore use of lesser amounts, of starting materials, as well as simplified separation and purification procedures, when employed as intermediates in the preparation of sidechain-bearing taxanes.

[0008] The present invention also provides novel methods for using the aforementioned β -lactams of the formula I in the preparation of sidechain-bearing taxanes, and the novel sidechain-bearing taxanes prepared.

[0009] The present invention is described further as follows.

[0010] The terms "alkyl" or "alk", as used herein alone or as part of another group, denote optionally substituted, straight and branched chain saturated hydrocarbon groups, preferably having 1 to 10 carbons in the normal chain. Exemplary unsubstituted such groups include methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl and the like. Exemplary substituents may include one or more of the following groups: halo, alkoxy, alkylthio, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, hydroxy or protected hydroxy, carboxyl (-COOH), alkyloxycarbonyl, alkylcarbonyloxy, carbamoyl (NH₂-CO-), amino (-NH₂), mono- or dialkylamino, or thiol (-SH).

[0011] The terms "lower alk" or "lower alkyl", as used herein, denote such optionally substituted groups as described above for alkyl having 1 to 4 carbon atoms in the normal chain.

[0012] The terms "alkoxy" or "alkylthio", as used herein, denote an alkyl group as described above bonded through an oxygen linkage (-O-) or a sulfur linkage (-S-), respectively. The term "alkyloxycarbonyl", as used herein, denotes an alkoxy group bonded through a carbonyl group. The term "alkylcarbonyloxy", as used herein, denotes an alkyl group bonded through a carbonyl group which is, in turn, bonded through an oxygen linkage. The terms "monoalkylamino" or "dialkylamino" denote an amino group substituted by one or two alkyl groups as described above, respectively.

[0013] The term "alkenyl", as used herein alone or as part of another group, denotes such optionally substituted groups as described for alkyl, further containing at least one carbon to carbon double bond. Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

[0014] The term "alkynyl", as used herein alone or as part of another group, denotes such optionally substituted groups as described for alkyl, further containing at least one carbon to carbon triple bond. Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

[0015] The term "cycloalkyl", as used herein alone or as part of another group, denotes optionally substituted, saturated cyclic hydrocarbon ring systems, preferably containing 1 to 3 rings and 3 to 7 carbons per ring. Exemplary unsubstituted such groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

[0016] The term "cycloalkenyl", as used herein alone or as part of another group, denotes such optionally substituted groups as described above for cycloalkyl, further containing at least one carbon to carbon double bond forming a partially unsaturated ring. Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

[0017] The terms "ar" or "aryl", as used herein alone or as part of another group, denote optionally substituted, homocyclic aromatic groups, preferably containing 1 or 2 rings and 6 to 12 ring carbons. Exemplary unsubstituted such groups include phenyl, biphenyl, and naphthyl. Exemplary substituents include one or more, preferably three or fewer, nitro groups, alkyl groups as described above, or groups described above as alkyl substituents.

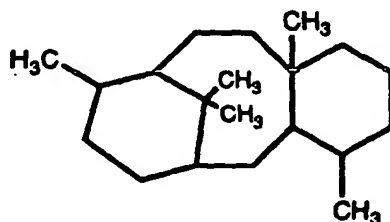
[0018] The term "arylcarbonyl", as used herein alone or as part of another group, denotes an aryl group as described above bonded through a carbonyl group.

[0019] The terms "heterocyclo" or "heterocyclic", as used herein alone or as part of another group, denote optionally substituted, fully saturated or unsaturated, aromatic or non-aromatic cyclic groups having at least one heteroatom in

at least one ring, preferably monocyclic or bicyclic groups having 5 or 6 atoms in each ring. The heterocyclo group may, for example, have 1 or 2 oxygen atoms, 1 or 2 sulfur atoms, and/or 1 to 4 nitrogen atoms in the ring. Each heterocyclo group may be bonded through any carbon or heteroatom of the ring system. Exemplary heterocyclo groups include the following: thienyl, furyl, pyrrolyl, pyridyl, imidazolyl, pyrrolidinyl, piperidinyl, azepinyl, indolyl, isoindolyl, quinolinyl, isoquinolinyl, benzothiazolyl, benzoxazolyl, benzimidazolyl, benzoxadiazolyl, benzofurazanyl, and especially, tetrahydropyranyl (e.g. 4-tetrahydropyranyl). Exemplary substituents include one or more alkyl groups as described above, or one or more groups described above as alkyl substituents.

[0020] The terms "halogen" or "halo", as used herein alone or as part of another group, denote chlorine, bromine, fluorine, and iodine.

[0021] The term "taxane moiety", as used herein, denotes moieties containing the core structure:



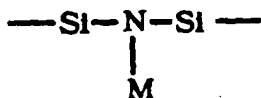
which core structure may be substituted and which may contain ethylenic unsaturation in the ring system thereof.

[0022] The term "taxane", as used herein, denotes compounds containing a taxane moiety as described above. The term "sidechain-bearing taxane", as used herein, denotes compounds containing a taxane moiety as described above, further containing a sidechain bonded to said moiety at C-13.

[0023] The term "hydroxy (or hydroxyl) protecting group", as used herein, denotes any group capable of protecting a free hydroxyl group which, subsequent to the reaction for which it is employed, may be removed without destroying the remainder of the molecule. Such groups, and the synthesis thereof, may be found in "Protective Groups in Organic Synthesis" by T.W. Greene, John Wiley and Sons, 1981, or Fieser & Fieser. Exemplary hydroxyl protecting groups include methoxymethyl, 1-ethoxyethyl, 1-methoxy-1-methylethyl, benzyloxymethyl, (β-trimethylsilylethoxy)methyl, tetrahydropyranyl, 2,2,2-trichloroethoxycarbonyl, t-butyl(diphenyl)silyl, trialkylsilyl, trichloromethoxycarbonyl, and 2,2,2-trichloroethoxymethyl.

[0024] The term "salt", as used herein, includes salts with organic and/or inorganic acids and/or bases.

[0025] The term "alkali metal silylamide base", as used herein, denotes a base containing the moiety:



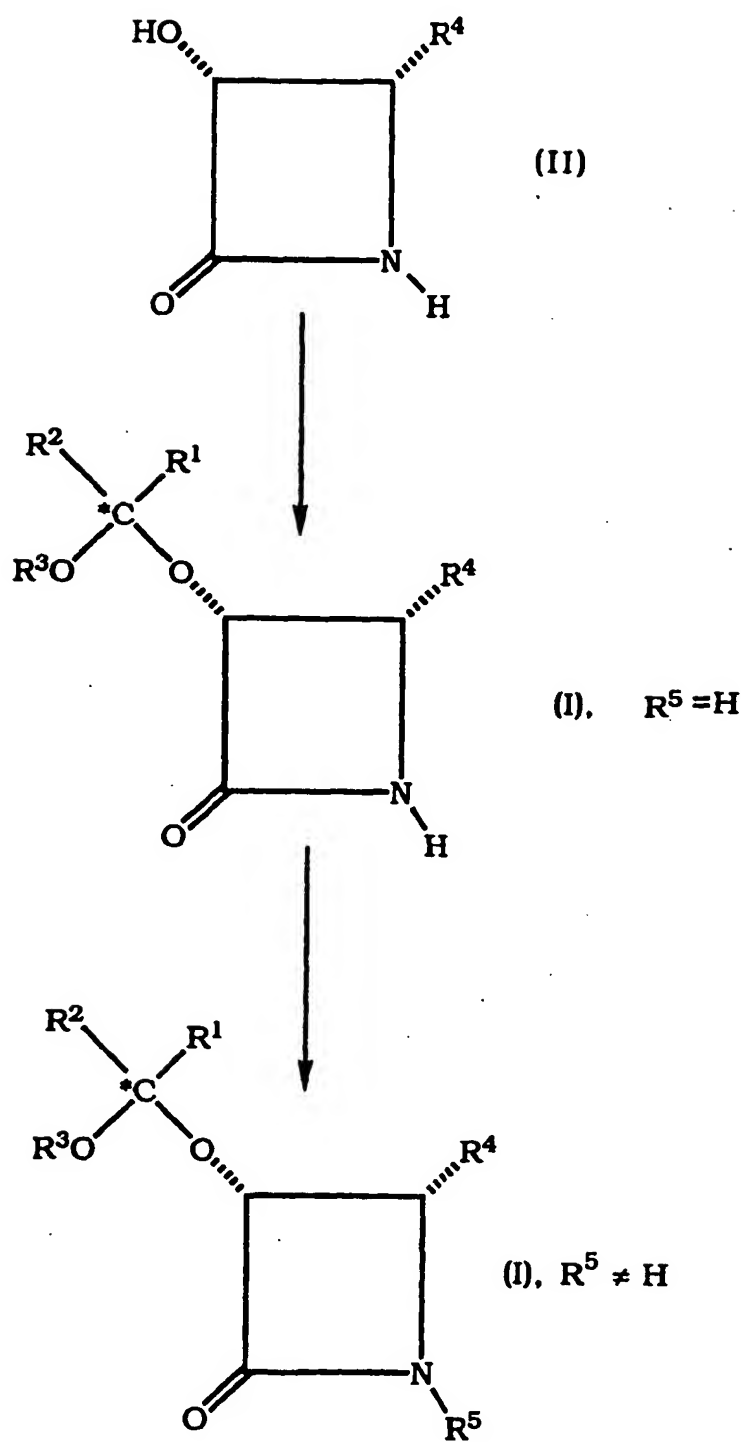
where M is an alkali metal such as lithium, sodium or potassium.

[0026] Preferred β-lactams of the present invention are those compounds of the formula I which are crystalline compounds, rather than liquids (oils) at ambient conditions. Such crystalline compounds are advantageous relative to liquid compounds as they may be more easily prepared and obtained in pure form, particularly at larger scales, thus facilitating their subsequent use as intermediates in the formation of sidechain-bearing taxanes such as taxol and taxol derivatives.

[0027] Particularly preferred compounds of the formula I are those where R¹ and R² are both the same unsubstituted lower alkyl group, especially where R¹ and R² are both methyl; R³ is unsubstituted lower alkyl, especially methyl; R⁴ is phenyl; and R⁵ is hydrogen, benzoyl or t-butoxycarbonyl.

[0028] β-lactams of the formula I may be prepared by methods such as those shown in the following Reaction Scheme for the preparation of cis β-lactams of the formula I.

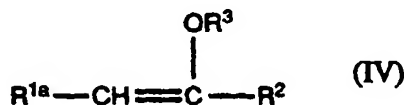
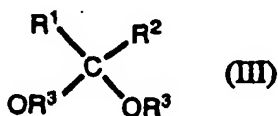
Reaction Scheme



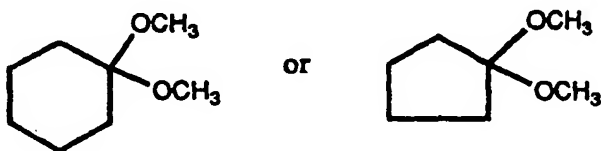
[0029] The starting compounds of the formula II may be prepared by methods such as those described in our Euro-

pean Patent Application No. EP-A-552,041, incorporated herein by reference. It is particularly preferred to employ (3-lactams which are stereoisomerically (that is, enantiomerically) pure.

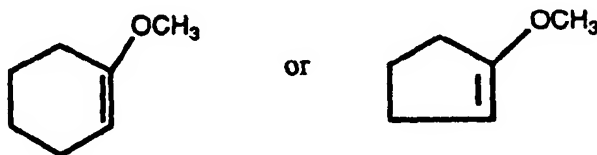
[0030] The compound of the formula II may be converted to a compound of the formula I by reaction of the former, in the presence of an acid catalyst, with a compound of the formula III or IV:



where R^1 , R^2 and R^3 are as defined above and R^{1a} (i) is a group such that $\text{R}^{1a}-\text{CH}_2-$ is the same as R^2 when R^2 is alkyl or (ii) forms, together with R^2 and the atoms to which R^{1a} and R^2 are bonded, a cycloalkenyl group or heterocyclo group containing at least one carbon to carbon double bond. Exemplary compounds of the formula III include the compounds: dimethoxypropane,



Exemplary compounds of the formula IV include the compounds:



[0031] A particularly preferred method for obtaining a compound of the formula I where R^1 and R^2 are both the same alkyl is by contacting a compound of the formula II with a compound of the formula IV where R^3 is as defined above and R^{1a} is a group such that $\text{R}^{1a}-\text{CH}_2-$ is the same as R^2 , in the presence of an acid catalyst such as an organic sulfonic acid, for example, pyridinium p-toluene sulfonate (PPTS), toluene sulfonic acid or camphor sulfonic acid. 2-Methoxypropene is preferred as the compound of the formula IV.

[0032] The aforementioned reaction is preferably conducted at a temperature of from about -30°C to about 30°C , especially at about 0°C , and at ambient pressure. The reaction may, for example, be completed over the course of about 0.5 hour to about 10 hours, and is preferably conducted under an atmosphere of inert gas such as argon.

[0033] Preferred mole ratios of the compound of the formula III or IV: the compound of the formula II are from about 6:1 to about 1:1. An amount of acid is employed which is effective to catalyze the reaction.

[0034] Organic solvents are preferably employed which are inert to the reaction. Particularly preferred solvents are acetone, dimethylformamide, tetrahydrofuran, dichloromethane, acetonitrile and toluene. Amounts of solvents are preferably those where the ratio of compound of the formula II: solvent is from about 1:5 to about 1:40, weight:volume.

[0035] The β -lactam of the formula I so obtained, where R^5 is hydrogen, may optionally be converted to a β -lactam of the formula I where R^5 is arylcarbonyl or alkyloxycarbonyl, with or without prior isolation of the β -lactam where R^5 is hydrogen, by contacting the former β -lactam where R^5 is hydrogen with a compound of the formula V or VI:



or



where

R^6 is aryl or alkoxy; and
 X is halo, especially chloro.

[0036] The above reaction is preferably conducted in the presence of a tertiary amine such as diisopropyl(ethyl) amine, triethylamine and 4-dimethylaminopyridine. Benzoyl chloride is preferred as the compound of the formula V, especially for the preparation of taxol. BOC anhydride (compound VI where R^6 is t-butoxy) is preferred as the compound of the formula VI, especially for the preparation of taxotere.

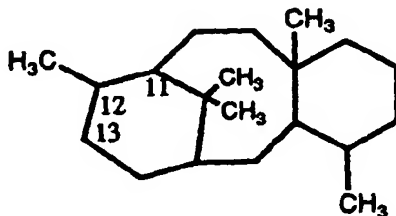
[0037] In the above reaction, it is preferred to employ temperatures of from about -30°C to about 30°C , especially about 0°C , and ambient pressure. The reaction may, for example, be completed over the course of about 2 hours to about 10 hours, and is preferably conducted under an atmosphere of inert gas such as argon.

[0038] Preferred mole ratios of the compound of the formula V or VI: β -lactam of the formula I where R^5 is hydrogen are from about 1:1 to about 5:1. Preferred mole ratios of tertiary amine: β -lactam of the formula I where R^5 is hydrogen are from about 1:1 to about 5:1.

[0039] Organic solvents are preferably employed which are inert to the reaction. Particularly preferred solvents are methylene chloride, tetrahydrofuran, acetonitrile, acetone, dimethylformamide and toluene. Amounts of solvents are preferably those where the starting β -lactam is from about 15% to about 80% by weight, based on the combined weight of solvent and starting β -lactam.

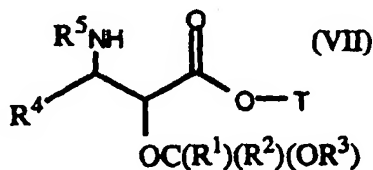
[0040] β -lactams where R^5 is not hydrogen are preferred for use in the coupling methods described following.

[0041] Taxanes are diterpene compounds containing the taxane moiety:



described above. Of particular interest are taxanes containing a taxane moiety in which the 11,12-positions are bonded through an ethylenic linkage, and in which the 13-position contains a sidechain, which taxanes are exemplified by taxol. Pharmacologically active taxanes such as taxol may be used as antitumor agents to treat patients suffering from cancers such as breast, ovarian, colon or lung cancers, melanoma and leukemia.

[0042] The present invention provides a novel method for the preparation of sidechain-bearing taxanes by coupling a β -lactam of the present invention to form said sidechain. In particular, the present invention provides a novel method for the preparation of a sidechain-bearing taxane of the following formula VII or a salt thereof:

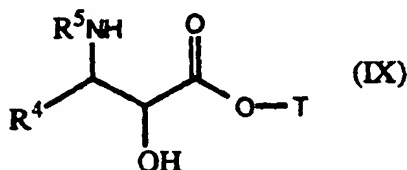


where R¹, R², R³, R⁴ and R⁵ are as defined above, and T is a taxane moiety bonded directly at C-13 of said moiety; comprising the step of contacting a β -lactam of the formula I or salt thereof of the present invention with a taxane compound of the following formula VIII or salt thereof:

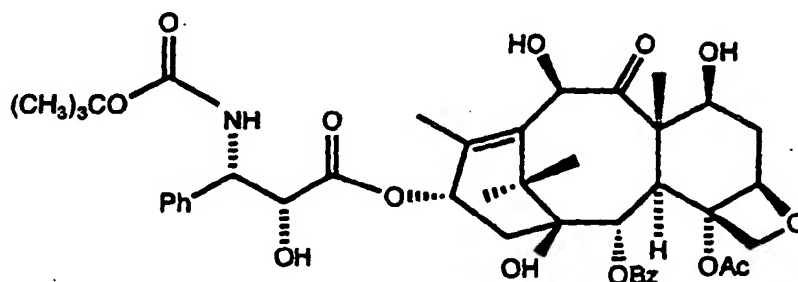
HO-T

(VIII)

where T is as defined above, in the presence of a coupling agent; and, optionally, converting the group -OC(R¹)(R²)(OR³) of said compound of the formula VII to hydroxyl, thereby forming a sidechain-bearing taxane or a salt thereof of the following formula IX:

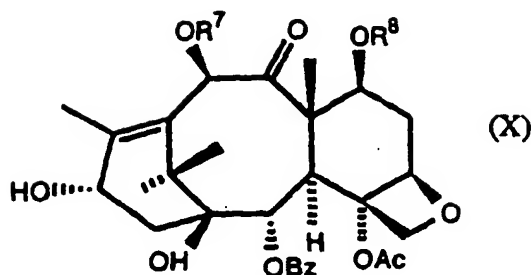


[0043] The addition of a sidechain as described above, in and of itself, may impart an increased or more desirable pharmacological activity to the taxane product, or may form a taxane product which is more readily converted to a taxane having an increased or more desirable pharmacological activity than the starting compound. Exemplary taxanes which may be prepared by the present method for the preparation of a sidechain-bearing taxane include those compounds described in European Patent Publication No. 400,971, U.S. Patent No. 4,876,399, U.S. Patent No. 4,857,653, U.S. Patent No. 4,814,470, U.S. Patent No. 4,924,012, and U.S. Patent No. 4,924,011, all incorporated herein by reference. It is preferred to prepare taxotere having the following structure:



or, most preferably, taxol as the compound of the formula IX.

[0044] Exemplary compounds of the formula VIII, having the OH group bonded directly therein at C-13, which may be employed in the method of the present invention are described in the aforementioned documents incorporated by reference, especially in European Patent Publication No. 400,971. Most preferably, the compound of the formula VIII is a compound of the formula X:



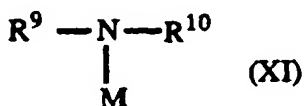
where

R⁷ is hydrogen, alkylcarbonyl, or a hydroxyl protecting group, especially acetyl; and
R⁸ is hydrogen or a hydroxyl protecting group;

and particularly is a 7-O-trialkylsilyl baccatin III such as 7-O-triethylsilyl baccatin III or 7-O-trimethylsilyl baccatin III. 7-O-triethylsilyl baccatin III may, for example, be obtained from 10-deacetyl baccatin III as described by Denis et al., *J. Am. Chem. Soc.*, **110**, 5917 (1988), incorporated herein by reference. 7-O-Triethylsilyl baccatin III is preferably prepared by the methods of the Examples herein. For example, ultimately, where R⁷ is hydrogen, compound (X) may be acylated *in situ* before sidechain coupling.

[0045] The coupling agent employed in the method of the present invention may be any agent facilitating coupling to form the sidechain-bearing taxane of the formula VII, exemplified by tertiary amines such as triethyl amine, diisopropyl (ethyl)amine, pyridine, N-methyl imidazole, and 4-dimethylaminopyridine (DMAP), and metallic bases allowing formation of a C-13 metal alkoxide on the taxane of the formula VIII such as lithium diisopropylamide (LDA), unsubstituted lower alkyl lithium compounds, or phenyllithium.

[0046] Preferably, the coupling agent of the present method is an alkali metal silylamide base or a sterically hindered alkali metal amide base. Exemplary such bases are those of the formula XI:



where

R⁹ and R¹⁰ are trialkylsilyl, cycloalkyl, or
together with the nitrogen atom to which they are bonded, form a heterocyclo group;
and
M is an alkali metal, such as lithium, sodium or potassium.

[0047] Preferred bases, particularly alkali metal silylamide bases of the formula XI, are those soluble in the reaction medium employed, and are most preferably an alkali metal hexamethyl disilazide (R⁹ and R¹⁰ are trimethylsilyl and M is sodium, lithium or potassium), especially lithium hexamethyldisilazide (LHMDS). "Sterically hindered alkali metal amide bases" include those bases containing the group -N(M)- where M is as defined above and which are substantially the same as, or more, sterically hindered than lithium hexamethyldisilazide in the coupling of a β -lactam to the C-13 hydroxyl group-containing taxane compound. Exemplary sterically hindered such bases include alkali metal tetramethyl piperidides and alkali metal dicyclohexylamides.

[0048] The aforementioned alkali metal bases, especially silylamide bases of the present method, are advantageous in that they are not strongly nucleophilic, so that degradation of the taxane starting material of the formula VIII is minimized or eliminated, and in that they provide a high yield (preferably, greater than or equal to about 90%) and purity (preferably greater than or equal to about 98%) of taxane product. The present invention further provides a method wherein a taxane of the formula VIII is coupled with any suitable β -lactam providing a sidechain at C-13 of said taxane, including but not limited to the β -lactams of the present invention, wherein an alkali metal silylamide base or

a sterically hindered metal amide base is employed as a coupling agent for said coupling.

[0049] The above coupling method of the present invention is preferably conducted at a temperature of from about -70°C to about 25°C, especially from about -30°C to about 0°C, and at ambient pressure. The reaction may, for example, be completed over the course of about one-half hour to about four hours, and is preferably conducted under an inert atmosphere such as argon.

[0050] Preferred mole ratios of taxane starting compound of the formula VIII: β -lactam are those greater than about 1:1.6, most preferably from about 1:1 to about 1:1.3, especially about 1:1.2. Preferred mole ratios of taxane starting compound of the formula VIII: alkali metal base, such as silylamide base, are from about 1:1.1 to about 1:1.5, especially about 1:1.1.

[0051] Organic solvents are preferably employed which are inert to the reaction. Particularly preferred solvents are tetrahydrofuran (THF), toluene and ether. Amounts of solvents are preferably those where the ratio of starting taxane of the formula VIII to solvent is from about 1:1 to about 1:5, preferably 1:2.5, weight:volume.

[0052] The method of the present invention further comprises, subsequent to the reaction forming a sidechain-bearing taxane of the formula VII, optionally converting the group $-\text{OC}(\text{R}^1)(\text{R}^2)(\text{OR}^3)$ to hydroxyl. These groups may optionally be converted to a hydroxyl group sequentially or simultaneously with other hydroxyl protecting groups, such as those on the taxane moiety, by suitable means, such as by contact with an acid, for example, an inorganic acid such as HCl or HF, or organic acids such as acetic acid and the like.

[0053] Preferably, deprotection is conducted at a temperature of from about -30°C to about 60°C, especially at about 0 to 25°C, and at ambient pressure. The reaction may, for example, be completed over the course of about 2 hours to about 72 hours, and is preferably conducted under an inert atmosphere such as argon.

[0054] Preferred mole ratios of acid for deprotection: taxane are from about 1:1 to about 20:1 (volume:weight). Organic solvents are preferably employed which are inert to the reaction. Particularly preferred solvents are an ethanol/tetrahydrofuran mixture or acetonitrile, acetone and water. Amounts of solvents are preferably those where the taxane is from about 1:10 to about 1:50, preferably 1:30, ratio of taxane: combined solvent, weight:volume (especially, tetrahydrofuran/ethanol and HCl/water).

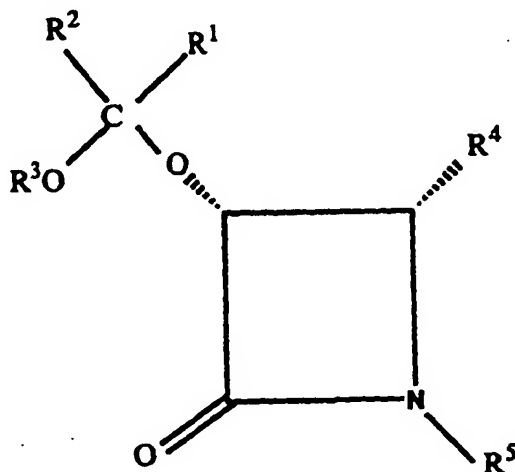
[0055] The present invention also provides the novel sidechain-bearing taxanes of the formula VII and salts thereof described herein.

[0056] Taxol is preferably ultimately prepared as the sidechain-bearing taxane by the methods of the present invention. Taxol may be prepared, for example, by contacting a 7-O-trialkylsilyl baccatin III such as 7-O-triethylsilyl baccatin III, as the formula VIII compound, with (3R-cis)-1-benzoyl-3-(1-methoxy-1-methylethoxy)-4-phenyl-2-azetidinone, as the β -lactam, preferably in the presence of an alkali metal silylamide base. The triethylsilyloxy and 1-methoxy-1-methylethoxy groups may be converted to hydroxyl groups subsequent to sidechain formation, by deprotection methods such as those described above, to form taxol.

[0057] Salts or solvates such as hydrates of reactants or products may be employed or prepared as appropriate in any of the methods of the present invention.

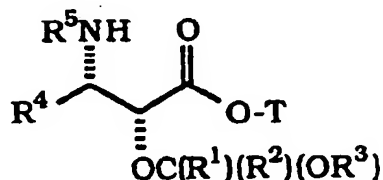
[0058] As can be appreciated, the β -lactams and taxanes described herein may be present in more than one stereoisomeric form. All stereoisomers of the compounds described herein are contemplated, either alone (i.e., substantially free of other isomers), or in admixture with other selected (e.g. as a racemate) or all other stereoisomers. It is preferred that these compounds be substantially free of other isomers, that is, enantiomerically pure.

[0059] Preferred stereoconfigurations of the compounds of the formula I are those where the groups $-\text{OC}(\text{R}^1)(\text{R}^2)(\text{OR}^3)$ and R^4 are in the cis position, that is,

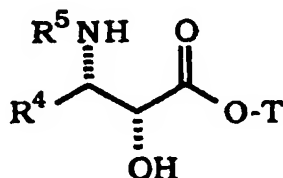


particularly where the compound of the formula I has the same absolute stereoconfiguration as the compound (3R-cis)-1-benzoyl-3-(1-methoxy-1-methylethoxy)-4-phenyl-2-azetidinone.

[0060] Preferred stereoconfigurations of the C-13 sidechains of the compounds of the formulae VII and IX correspond to the stereoconfiguration of the aforementioned cis β -lactams, that is,



and



which sidechains have the same absolute stereoconfiguration as that of taxol.

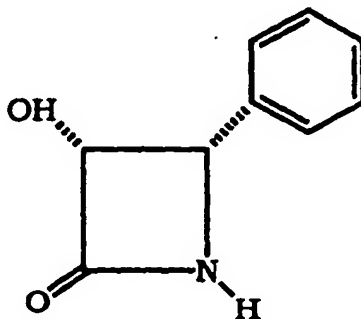
[0061] The present invention is further described by the following examples which are illustrative only.

Example 1

Preparation of (3R-cis)-3-(1-Methoxy-1-methylethoxy)-4-phenyl-2-azetidinone

(a) (3R-cis)-3-Hydroxy-4-phenyl-2-azetidinone

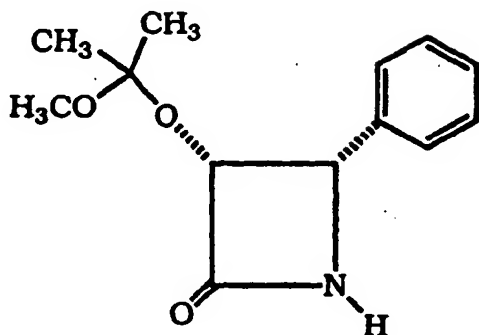
[0062]



[0063] The title compound was prepared by enzymatic hydrolysis of racemic 3-acetyloxy-4-phenyl-2-azetidinone (see U.S. Application Serial No. 07/822,015, filed January 15, 1992 by Patel et al.) to form (3R-cis)-3-acetyloxy-4-phenyl-2-azetidinone, followed by hydrolysis using base to form the optically active title compound.

(b) (3R-cis)-3-(1-Methoxy-1-methylethoxy)-4-phenyl-2-azetidinone

[0064]



[0065] The product of step (a) above (8.49 g, 52.0 mmol) was added to a dry 500 ml 3-necked flask (dried in a 120°C oven for ~12 hours and equipped with a magnetic stirbar and a digital thermometer), purged with argon, and dissolved in acetone (300 ml, freshly opened bottle of HPLC grade acetone; wt % H₂O (K.F.) <0.001). The yellowish solution was cooled to 0° (internal temperature was 1°C). 2-Methoxypropene (15.0 ml, 156 mmol) (wt. % H₂O (K.F.) <0.001) was added dropwise over a period of 30 seconds. The internal temperature rose to ~2°C during the addition of 2-methoxypropene. The resulting solution was stirred at 0°C for 5 minutes before the addition of pyridinium *p*-toluene sulfonate (PPTS) (1.3 g, 5.2 mmol) (wt. % H₂O (K.F.) = 0.001). After stirring at 0°C for 30 minutes, TLC (thin layer chromatography) analysis revealed that the reaction was complete. (TLC analysis (silica gel, solvent: ethyl acetate, stain: phosphomolybdic acid/ethanol) of the crude reaction revealed a spot for the product (*R*_f = 0.50) and no starting material (*R*_f = 0.31)).

[0066] The solution was combined with ethyl acetate (250 ml), saturated aqueous NaHCO₃ (200 ml), and H₂O (100 ml) in a separatory funnel. After shaking the mixture and separating the layers, the aqueous fraction was extracted with ethyl acetate (3 x 100 ml). The combined organic fractions were washed with saturated aqueous NaCl (200 ml), dried over Na₂SO₄, filtered, and concentrated on a rotovap to give an off-white solid. All concentrations on the rotovap were conducted with a bath temperature of 35°C.

[0067] The crude product was dissolved in ethyl acetate (200 ml) and neutral activated charcoal (2 g) was added. The mixture was boiled gently for 5 minutes, cooled to room temperature, and suction filtered through a pad of Celite. Removal of the solvent on a rotovap as above, followed by exposure to high vacuum (~133Pa, ~1 mm Hg for 45 minutes) gave 11.9 g of an off-white solid. The solid was dissolved in boiling ethyl acetate (75 ml), and boiling hexanes (400 ml) were then added in 50 ml portions. The resulting cloudy solution was allowed to cool to room temperature. Crystallization began within ~1 minute after the solution was removed from the heat source. After standing at room temperature for 45 minutes, the mixture was chilled in a 4°C cold room for 15 hours. The crystals were filtered, washed with 1:19 ethyl acetate/hexanes (3 x 100 ml) on a suction filter, and dried under high vacuum (~20Pa, ~0.15 mm Hg for 20 hours) to give 9.55 g (78%) of the title product as off-white needles.

[0068] The mother liquor was concentrated on a rotovap as above, exposed to high vacuum (~133Pa, ~1 mm Hg for 0.5 h.), and was then crystallized from ethyl acetate/hexanes to give 1.48 g (12%) of small off-white crystals of the title product. (The crystallization was performed in a similar manner as that for the first crop. The solid was dissolved in 5 ml of boiling ethyl acetate, and boiling hexanes (~40 ml) were added in ~5 ml portions until a few crystals appeared. Crystallization began immediately upon cooling to room temperature. The mixture was allowed to stand at room temperature for 1.5 h., then at 4°C for 16 hours. The crystals were filtered, washed with 3 x 25 ml 1:19 ethyl acetate/hexanes on a suction filter, and dried under high vacuum (~0.2 mm Hg) for 24 hours).

For title product:

Elemental Analysis (%) C ₁₃ H ₁₇ NO ₃		
	Calcd.	Found
C	66.36	66.30
H	7.28	7.40

(continued)

Elemental Analysis (%) C ₁₃ H ₁₇ NO ₃		
	Calcd.	Found
N	5.95	6.04
H ₂ O (KF)	0.00	0.00

m.p. 136 - 137°C

[α]_D²²: +6.7° (c 1.0, CHCl₃)[α]₃₆₅²²: +93.3° (c 1.0, CHCl₃)TLC: R_f = 0.47 (silica gel, ethyl acetate) visualized by phosphomolybdic acid/ethanol.Example 2Preparation of (3R-cis)-3-(1-Methoxy-1-methylethoxy)-4-phenyl-2-azetidinone

[0069] The title product of step (a) of Example 1 above (30.1 g, 184 mmol, having a brownish color) was added to a flame-dried, argon-purged 500 mL flask (the flask was dried in a 120°C oven for ~12 h. and was equipped with a magnetic stirbar and a digital thermometer), and dissolved in dimethylformamide (300 mL, wt. % H₂O (K. F.) = 0.05). The reddish-brown solution was cooled to 0°C. The internal temperature was 2°C.

2-Methoxypropene (53.0 mL, 553 mmol) was added dropwise over a period of 2 minutes (the internal temperature rose to ~2°C during the addition of 2-methoxypropene), and the resulting solution was stirred at 0°C for 5 minutes before the addition of pyridinium p-toluene sulfonate (PPTS, 4.6 g, 18.4 mmol). Approximately 5 minutes after the PPTS addition, the reaction temperature reached a maximum of 4.8°C. The solution became lighter in color as the reaction progressed. After stirring at 0°C for 1 h, TLC analysis revealed that the reaction was complete. (TLC analysis (silica gel, solvent: ethyl acetate, stain: phosphomolybdic acid/ethanol) of an aliquot partitioned between ethyl acetate and H₂O revealed a spot for the product (R_f = 0.51) and no starting material (R_f = 0.33)).

[0070] The solution was diluted with a 3:1 ethyl acetate/hexanes mixture (600 mL) and washed with half-saturated aqueous NaHCO₃ (500 mL). During the NaHCO₃ wash, most of the colored impurity was extracted into the aqueous phase. However, the organic phase remained a reddish-brown color. The aqueous fraction was extracted with ethyl acetate (2 x 150 mL). The combined organic fractions were washed with H₂O (500 mL) (TLC analysis of the H₂O wash showed no loss of the product to the aqueous layer), saturated aqueous NaCl (200 mL), dried over Na₂SO₄, filtered, and concentrated on a rotovap to give an off-white solid. All concentrations on the rotovap were conducted with a bath temperature of 40°C. The solid was dissolved in boiling ethyl acetate (180 mL), and hexanes (250 mL) were then added in ~20 mL portions until a few crystals appeared. The resulting solution was removed from the heat source and allowed to cool to room temperature. Extensive crystallization began within ~1 minute after the solution was removed from the heat source. After standing at room temperature for 1 h, the mixture was chilled in a 4°C cold room for 17 h. The crystals were filtered, washed with 1:19 ethyl acetate/hexanes (3 x 150 mL) on a suction filter, and dried under high vacuum (~66Pa, ~0.5 mm Hg for 22 h.) to give 32.6 g (75.4%) of the title product as fluffy white needles.

[0071] The mother liquor was concentrated on a rotovap as above, and was then crystallized from ethyl acetate/hexanes to give 6.25 g (14.4%) of the title product as fluffy white crystals. The crystallization was performed in a similar manner as that for the first crop. The solid was dissolved in 25 mL of boiling ethyl acetate, and hexanes (~60 mL) were added in ~5 mL portions until a few crystals appeared. Crystallization began immediately upon cooling to room temperature. The mixture was allowed to stand at room temperature for 1 h, then at 4°C for 14 h. The crystals were filtered, washed with 3 x 100 mL 1:19 ethyl acetate/hexanes on a suction filter, and dried under high vacuum (~80Pa, ~0.6 mm Hg) for 16 h. (Yield = 90%).

Elemental Analysis (%) C ₁₃ H ₁₇ NO ₃		
	Calcd.	Found
C	66.36	66.40
H	7.28	7.20
N	5.95	5.68
H ₂ O (KF)	0.00	0.00

m.p. = 141°C

$[\alpha]_D^{22}$: +6.5° (c 1.0, CHCl_3)

$[\alpha]_{365}^{22}$: +95.0° (c 1.0, CHCl_3)

TLC: R_f = 0.47 (silica gel, ethyl acetate) visualized by phosphomolybdic acid/ethanol.

[0072] The following alternative procedures were employed to prepare the title product:

(1) A mixture of the title product of step (a) of Example 1 (79.7 mg, 0.488 mmoles), dimethoxy propane (0.3 ml, 2.44 mmol), PPTS (about 12 mg, 0.049 mmol) and dimethylformamide (2 ml) under argon were stirred for 3 hours at about 0°C and then for 24 hours at about 4°C. The product obtained was extracted with ethyl acetate and worked up (diluted with 10 ml ethyl acetate, washed with 0.5 saturated aqueous NaHCO_3 ; aqueous fraction extracted with 2x5 ml ethyl acetate; combined organic fractions were washed with 1x10 ml water, 10 ml saturated aqueous NaHCO_3 , dried over Na_2SO_4 , filtered and concentrated) to obtain quantitative yield of products, which was a 1:1.7 mixture of starting material and the title product (determined by TLC analysis).

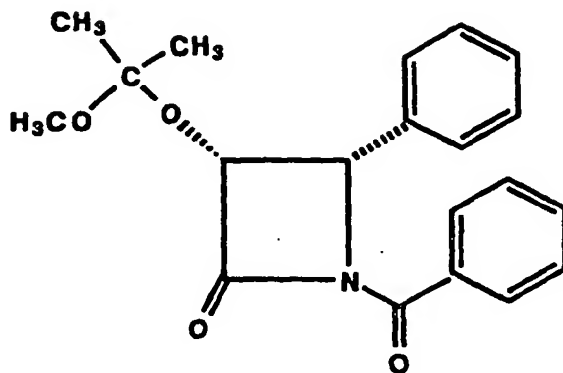
(2) The title product was obtained by adding the title product of step (a) of Example 1 (92.5 mg) to an oven-dried 5 ml flask, purged with argon, diluted with dimethylformamide (1.5 ml) and cooled to 0°C. Dimethoxy propane (0.18 g) was added, followed by PPTS (14 mg). The solution was stirred at 0°C for 5 hours, and worked up as above (yielding about 1:1.1 starting material to title product).

(3) The title product was obtained by adding the title product of step (a) of Example 1 (89.4 mg) to an flame-dried, argon-purged flask, dissolved in acetone (3.5 ml) and cooled to 0°C. Dimethoxy propane (0.17 g) was added, followed by PPTS (14 mg). The solution was stirred at 0°C for 3 hours, transferred to a 4°C cold room for 24 hours, and worked up to yield the title product in about a 8:2:1 starting material to title product to impurity ratio.

Example 3

Preparation of (3R-cis)-1-Benzoyl-3-(1-methoxy-1-methylethoxy)-4-phenyl-2-azetidinone

[0073]



[0074] The title product of step (b) of Example 1 above (8.69 g, 36.9 mmol) was added to a dry 250 mL 3-necked flask (dried in a 120°C oven for 24 hours and equipped with a magnetic stirbar and a digital thermometer), purged with argon and dissolved in CH_2Cl_2 (90 mL) (wt. % H_2O (K.F.) <0.05). Diisopropyl(ethyl)amine ($i\text{-Pr}_2\text{NEt}$, 7.10 mL, 40.6 mmol) (wt. % H_2O (K.F.) = 0.016) was added over a period of 30 seconds and then 4-dimethylaminopyridine (0.90 g, 7.4 mmol) (wt. % H_2O (K.F.) <0.05) was added in one portion. The resulting solution was cooled to 0°C (the internal temperature was measured at 1°C) and benzoyl chloride (4.70 mL, 40.6 mmol) was then added dropwise over a period of 7 minutes. The internal temperature rose to 8°C during the addition. A slightly cloudy solution was obtained after the addition, which became a clear yellowish solution upon stirring at 0°C. The solution was then stirred at 0°C for 1.5 h, at which time TLC analysis showed the reaction to be complete. (TLC analysis (silica gel, solvent: ethyl acetate, stain: phosphomolybdic acid/ethanol) of the crude reaction revealed a spot for the product (R_f = 0.61) and no starting material (R_f = 0.49).)

[0075] The solution was diluted with CH_2Cl_2 (150 mL), washed with saturated aqueous NaHCO_3 , and the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (75 mL). The combined organic layers were washed with 5.7% aqueous NaH_2PO_4 (300 mL; measured pH of 5.7% aqueous NaH_2PO_4 = 4.25 ± 0.05 ; measured pH of the resulting washing = 5.57 ± 0.05), saturated aqueous NaCl (100 mL), dried over Na_2SO_4 , filtered, and concentrated on

a rotovap to give an off-white foam. All concentrations on the rotovap were conducted with a bath temperature of 35°C. The crude product was dissolved in ethyl acetate (150 mL) and neutral activated charcoal (2 g) was added. The resulting mixture was boiled gently for 5 minutes, cooled to room temperature, and suction-filtered through a pad of Celite. The solution was considerably less colored than before the charcoal treatment. Removal of the solvent on a rotovap as above, followed by trituration of the resulting foam with hexanes (50 mL) gave a slurry of the solid product.

[0076] The slurry was concentrated on a rotovap as above and exposed to vacuum (~2 mm Hg for 15 minutes) to give 12.2 g of an off-white solid. The solid was dissolved in hot ethyl acetate (7 mL) and hot hexanes (~45 mL) were added in ~2 mL portions. This crystallization was conducted carefully to avoid having the product oil out. The resulting cloudy solution was then removed from the heat source. After a few minutes of cooling, a seed crystal was added and crystallization began within 10 minutes. After 1 hour at room temperature, the mixture was placed in a 4 °C cold room for 4 hours. The crystals were then filtered, washed with 1:19 ethyl acetate/hexanes (3 x 50 mL) on a suction filter, and dried under high vacuum (~0.2 mm Hg for 16 hours) to give 9.23 g (73.7%) of the title product as off-white crystals. The mother liquor contained additional product (by TLC analysis), but a second crop was not crystallized.

Elemental Analysis (%) C ₂₀ H ₂₁ NO ₄ • H ₂ O		
	Calcd.	Found
C	70.41	70.14
H	6.26	6.10
N	4.11	4.13
H ₂ O (KF)	0.53	0.55

m.p. 89 - 94°C

[α]_D²²: +173.1° (c 1.0, CHCl₃)

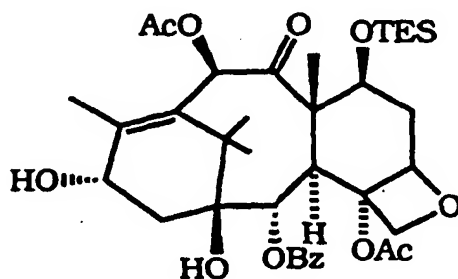
TLC: R_f = 0.58 (silica gel, ethyl acetate) visualized by phosphomolybdic acid/ethanol.

Example 4

Preparation of [2aR-[2aα,4β,4aβ,6β,9α(αR*,βS*),-11α, 12α, 12aα, 12bα]]-β-(Benzoylamino)-α-(1-methoxy-1-methylethoxy)hydroxybenzenepropanoic acid, -6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a, 5,6,9,10,11,12,12a, 12b-dodecahydro-4-triethylsilyloxy-11-hydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl-ester

(a) 7-TES Baccatin III

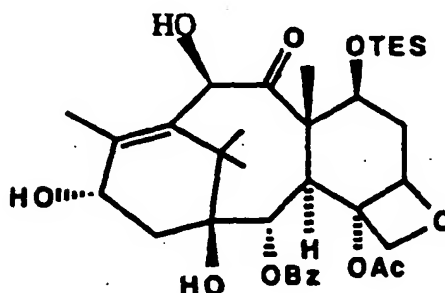
[0077]



[0078] As used herein, Ac is acetyl, Bz is benzoyl and TES is triethylsilyl.

(i) [2aR-(2a α ,4 β ,6 β ,9 α ,11 β ,12 α ,12a α ,12b α)]-Benzoic acid, 12b-acetyloxy-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-6,9,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-4-[(triethylsilyl)oxy]-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-12-yl ester (7-O-TES-10-Desacetyl baccatin III)

[0079]



[0080] 10-Desacetyl baccatin III (27.4 g, 50.3 mmol) (amount not corrected for impurities measured (twice) as: H₂O: 1.0% (1.57%), CH₃OH: 1.49% (1.6%), ethyl acetate: 0.1% (0.09%), hexane (0.03%)) and 4-dimethylaminopyridine (2.62 g, 21.4 mmol) (wt. % H₂O (K.F.) = 0.09) were added to a flame-dried, argon purged 1 L 3-necked flask (equipped with a mechanical stirrer and a digital thermometer) and were dissolved in dry dimethylformamide (122 ml) (wt. % H₂O (K.F.) = <0.01). CH₂Cl₂ (256 ml) (wt. % H₂O (K.F.) = <0.01) was added and the resulting homogeneous solution was cooled to -50°C. (The temperature of the reaction solution rose from 23°C to 25°C during the addition of CH₂Cl₂.) Triethylamine (NEt₃, 16 ml, 120 mmol) (wt. % H₂O (K.F.) = 0.08) was added dropwise over 3 minutes and the resulting solution was stirred at -50°C for 5 minutes before the dropwise addition of neat triethylsilyl chloride (Et₃SiCl, 18.6 ml, 111 mmol). The addition of Et₃SiCl was conducted over a period of 10 minutes and the temperature of the reaction did not rise above -50°C. The reaction became very cloudy during the addition of Et₃SiCl. The resulting mixture was stirred at -50°C for 1 hour and was then allowed to stand (without stirring) in a -48°C freezer for 22 hours. (A separate experiment showed that stirring the reaction at -48°C for 8 hours resulted in ~60% conversion.) The mixture was then removed from the freezer and warmed to ~-10°C. (TLC analysis of the mixture (solvent: ethyl acetate, stain: phosphomolybdic acid/ethanol) revealed the absence of starting material and showed a single spot for the product (R_f = 0.60).) The cold mixture was combined with EtOAc (1L) and washed with H₂O (890 ml). The resulting aqueous layer was separated and extracted with EtOAc (250 ml). The combined organic layers were washed with 5.7% aqueous NaH₂PO₄ (2 x 250 ml) (measured pH of 5.7% aqueous NaH₂PO₄ = 4.30 ± 0.05; measured pH of the combined NaH₂PO₄ washings = 5.75 ± 0.05), half-saturated aqueous NaCl (250 ml), saturated aqueous NaCl (250 ml), dried over Na₂SO₄, filtered and concentrated on a rotovap. (All concentrations on the rotovap were conducted with a water bath temperature of 35°C.) The resulting semi-solid was further dried by exposure to high vacuum (~133Pa, ~1 mm Hg for 20 minutes) to give 41.5 g of a white solid. The crude product was then dissolved in CH₂Cl₂ (400 ml) (heating in a 35°C water bath was required to dissolve the solid) and the volume of the resulting solution was reduced to ~150 ml on a rotovap. Crystallization started immediately and the mixture was allowed to stand at room temperature for 1 hour. Hexanes (100 ml) were added and the mixture was gently swirled. The mixture was allowed to stand in a 4°C cold room for 16.5 hours. The solid was filtered, washed with 1:9 CH₂Cl₂/hexanes (3 x 250 ml) on a suction filter, and dried under high vacuum (~27Pa, ~0.2 mm Hg for 42 hours) to give 26.1 g (79%) of the title product as a white powder. The mother liquor was concentrated on a rotovap and the residue was crystallized from CH₂Cl₂ to give 4.5 g (14%) of the title product as white crystals. Recrystallization was conducted in the same manner as with the first crop of product: the solid was dissolved in CH₂Cl₂ (100 ml) without heating and the volume of the resulting solution was reduced to ~7 ml on a rotovap. Crystallization began within 5 minutes. The mixture was allowed to stand at room temperature for 1 hour, then in a 4°C cold room for 42 hours. The crystals were filtered, washed with 1:9 CH₂Cl₂/hexanes (3 x 50 ml) on a suction filter, and dried under high vacuum (~27Pa, ~0.2 mm Hg for 18 hours.). The ¹H NMR of this crop was identical to the ¹H NMR of the first crop of product.

[0081] The combined yield for the two crops was 93% (uncorrected).

Elemental Analysis (%) C ₃₅ H ₅₀ O ₁₀ Si		
	Calcd.	Found
C	63.80	63.43
H	7.65	7.66
KF(H ₂ O)	0.00	0.00

mp: 239 - 242°C (decomp.)

$[\alpha]_D^{22}$: -53.6° (c 1.0, CHCl₃)

TLC: R_f = 0.60 (silica gel, EtOAc); visualized by phosphomolybdic acid/ethanol.

[0082] An alternative procedure was employed as follows:

[0083] In a flame-dried 250 ml 3-necked flask equipped with an argon inlet was placed 10-desacetyl-baccatin III (5.44 g, 10 mmol, having a water content of 1.56 wt. % and a methanol content of 1.6 wt %), 4-dimethylaminopyridine (0.49 g, 4 mmol) and N,N-dimethylformamide (24 ml, dried over 4Å molecular sieve). The mixture was stirred at room temperature until homogeneous.

Dichloromethane (50 ml, HPLC grade, used without purification) was added and the temperature was lowered to -50°C. Triethylamine (2.9 ml, 21 mmol) was added dropwise over a 5 minute period, followed by triethylsilylchloride (3.4 ml, 20 mmol) over a 10 minute period. The mixture was allowed to stand at -48°C for a period of 21 hours, diluted with 200 ml of ethyl acetate and 175 ml of water. (The reaction was monitored by TLC using EtOAc as eluent: R_f for the starting material = 0.56, R_f for the product = 0.83; UV and PMA visualization.) The aqueous layer was separated and extracted with ethyl acetate (50 ml x 1). The organic layers were combined and washed with 5% aqueous potassium phosphate mono basic (50 ml x 2) (pH of 5% KH₂PO₄ in H₂O was 4.3), half-saturated sodium chloride (50 ml x 1), brine (50 ml x 1), dried over sodium sulfate and concentrated *in vacuo* to give crude title product as a solid (7.45 g). The crude material was dissolved in 75 ml of hot dichloromethane and the total volume was reduced to 30 ml by heating to begin crystallization. It was set aside at room temperature for 2 hours and 4°C for 16 hours. The crystals were filtered on a buchner funnel, washed with cold 10% dichloromethane in hexane (25 ml) and dried *in vacuo* to afford 5.38 g of title product. The mother liquors and washings were concentrated *in vacuo* and the solid residue was crystallized by dissolving in 8 ml of dichloromethane. Following the above crystallization procedure, 0.72 g of the product was obtained as a second crop. The combined yield of the title product 7-TES-10-desacetyl baccatin III, as a white solid, m.p. 238-240°C was 6.10 g (93%).

Elemental Analysis (%) C ₃₅ H ₅₀ O ₁₀ Si		
	Calcd.	Found
C	63.80	63.76
H	7.65	7.66

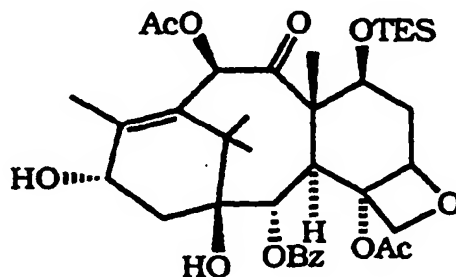
mp: 239 - 242°C

$[\alpha]_D$: -53.7 (c 1.0, CHCl₃)

TLC: R_f = 0.53 (silica gel, 50% EtOAc in hexane); UV and PMA visualization. HI = 98.9%

(ii) [2aR-(2a α ,4 β ,4a β ,6 β ,9 α ,11 β ,12 α ,12a α ,12b α)]-6,12b-Bis(acetyloxy)-12-(benzoyloxy)-2a,3,-4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-9,11-dihydroxy-4a,8,13,13-tetramethyl-4-[(triethylsilyl)oxy]-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-5-one (7-O-TES-baccatin III)

[0084]



[0085] 7-O-TES-10-desacetyl baccatin III prepared in step (i) above (21.4 g, 32.4 mmol) was added to a flame-dried, argon purged 1L 3-necked flask (equipped with a mechanical stirrer and a digital thermometer) and dissolved in THF (350 ml, freshly distilled from sodium/benzophenone). The resulting solution was cooled to -70°C. A solution of n-butyllithium (n-BuLi, 14.6 ml of a 2.56 M solution in hexanes, 37.3 mmol, titrated in triplicate with diphenylacetic acid in THF at 0°C) was added dropwise over a period of 23 minutes. The temperature of the reaction did not rise above -68°C during the addition. Solids were formed upon the addition of n-BuLi and did not appear to dissolve at -70°C. The resulting mixture was stirred at -70°C for 20 minutes and was then warmed to -48°C. (A clear homogeneous solution was obtained upon warming to -48°C.) After stirring at -48°C for 1/2 hour, acetic anhydride (4.6 ml, 49 mmol, distilled (137 - 138°C, 1 atm) under an atmosphere of argon before use) was added dropwise over 7 minutes. The temperature of the reaction did not rise above -45°C during the addition. The resulting solution was stirred at -48°C for 20 minutes and then at 0°C for 1 hour. The solution was diluted with ethyl acetate (350 ml), washed with saturated aqueous NH₄Cl (250 ml), and the layers were separated. The aqueous layer was extracted with ethyl acetate (200 ml). The combined organic layers were washed with saturated aqueous NaCl, dried over Na₂SO₄, filtered, and concentrated on a rotovap. (All concentrations on the rotovap were conducted with a water bath temperature of 35°C.) Exposure of the semi-solid to high vacuum (~200Pa, ~1.5 mm Hg for 1/2 hour) gave 24.7 g of a white solid. The crude product was dissolved in CH₂Cl₂ (300 ml) and the volume of the resulting solution was reduced to ~70 ml on a rotovap. Crystallization began within one minute. The mixture was allowed to stand at room temperature for 45 minutes, and then in a 4°C cold room for 18 hours. The crystals were filtered, washed with 1:9 CH₂Cl₂/hexanes (3 x 100 ml) on a suction filter, and dried under high vacuum (~27Pa, ~0.2 mm Hg for 19 hours) to give 20.9 g (92.0%) of the title product as fine white needles. The mother liquor was concentrated on a rotovap and the residue was crystallized from CH₂Cl₂/hexanes to give 0.82 g (3.6%) of the title product as small white crystals. Crystallization was conducted as follows: The residue was dissolved in CH₂Cl₂ (10 ml) and the volume of the resulting solution was reduced to ~5 ml on the rotovap. After standing at room temperature for 1/2 hour, no crystals had formed. Hexanes (5 ml) were added in 1 ml portions and solution was swirled. A few crystals were present by this time. The mixture was allowed to stand at room temperature for 1/2 hour (more crystals formed) and then in a 4°C cold room for 18 hours. The crystals were filtered, washed with 1:9 CH₂Cl₂/hexanes on a suction filter, and dried under high vacuum (~20Pa, ~0.15 mm Hg for 21 hours).

[0086] The combined yield for the two crops was 95.6%.

mp: 218 - 219°C (decomp.)

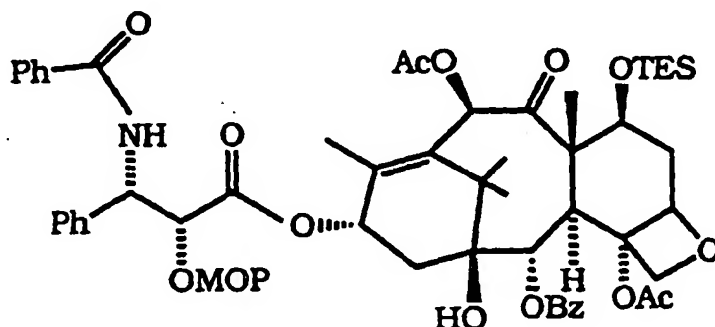
[α]_D²²: -78.4° (c 1.0, CHCl₃)

TLC: R_f = 0.37 (silica gel, 1:9 acetone/CH₂Cl₂); visualized by phosphomolybdic acid/ethanol.

(b) [2aR-[2a α ,4 β ,4a β ,6 β ,9 α (α R*, β S*),11 α ,12 α ,12a α ,12b α]]- β -(Benzoylamino)- α -(1-methoxy-1-methylethoxy)hydroxybenzenepropanoic acid, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,-12a,12b-

dodecahydro-4-triethylsilyloxy-11-hydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz
[1,2-b]-oxet-9-yl-ester

[0087]



[0088] As used herein, Ph is phenyl, MOP is 1-methoxy-1-methylethyl, and THF is tetrahydrofuran.

[0089] To a solution of the compound prepared in step (a) above (50.00 g, 71.33 mmol) in THF (freshly distilled from sodium and benzophenone, 125 ml) at -50°C (the cooling was applied only after the compound was completely dissolved in THF) was added dropwise with vigorous stirring lithium hexamethyldisilazide (LHMDS, 55.1 ml, 1.36 M in THF, 74.90 mmol; the reagent was titrated with 1,3-diphenylacetone p-tosylhydrazine) over a period of 20 minutes, so that the internal temperature did not rise above -48°C. After the addition the reaction mixture was warmed to -35°C and stirred at that temperature for 5 minutes.

[0090] A freshly prepared solution of the compound prepared as the title product of Example 3 ("Compound 3") (27.85 g, 82.03 mmol) in THF (35 ml) was added dropwise to the reaction mixture over a period of 7 minutes. No significant exotherm was observed. The flask containing Compound 3 was washed with 5 ml of THF and the washing transferred to the reaction mixture. The resulting solution was brought to 0°C by replacing the dry-ice bath with an ice-water bath and stirred for an additional 90 minutes. The reaction was monitored by TLC on reverse phase silica gel (EM Science RP-18 WF₂₅₄S) using acetonitrile/water (70/30) as eluent. R_f for the title product was 0.31, for 7-TES-taxol (that is, the structure of taxol in which the 7-position hydroxyl group is replaced with TES-O-) 0.41, for 7-TES-baccatin III 0.47, for Compound 3, 0.63.

[0091] The reaction was quenched with a pH 7 phosphate buffer (50 ml), followed immediately by saturated NaHCO₃ (150 ml). It was diluted with ethyl acetate (EtOAc, 600 ml) and the layers were separated. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give the crude title product (82.3 g) as a pale yellow solid. The solid was dissolved in hot EtOAc (200 ml) and hexanes (110 ml) were added dropwise at the reflux temperature. The crystallization mixture was set aside at room temperature for 2 hours (upon cooling precipitation occurred rapidly), and then in a cold room for 7 hours. The solid was filtered and washed with a cold mixture of hexanes/EtOAc, 5/1 (2 x 80 ml). The resulting white crystals were dried on the suction filter for 1 hour, and then *in vacuo* (~80 Pa, ~0.6 mm Hg) overnight to give 67.37 g of the title product (91% based on 7-TES-baccatin III; ¹H NMR showed 0.4 mol of EtOAc which gave a corrected yield of 87%) with an effective homogeneity index (HI) of 99.25% (95.73% title product and 3.52% 7-TES-taxol).

[0092] The mother liquor and the washings were combined and evaporated to dryness. The residue was dissolved in hot EtOAc (25 ml) and hexanes (40 ml) were added dropwise at the reflux temperature. After cooling to room temperature the mixture was set aside at room temperature for 1 hour, followed by 7 hours in the cold room. The solid was collected by filtration, dried on a suction filter and then *in vacuo* overnight (~93 Pa, 0.7 mm Hg) to yield 6.06 g (8%) of the title product with an effective HI of 96.6% (92.6% title product and 4.0% 7-TES-taxol.)

Elemental Analysis (%) C ₅₇ H ₇₃ NO ₁₅ Si • 0.4 EtOAc		
	Calcd.	Found
C	65.44	65.49
H	7.14	7.44
N	1.30	1.47

The resulting white crystals were dried on the suction filter for 20 minutes, and then *in vacuo* (~67Pa, ~0.5 mm Hg) overnight to give 6.59 g of the title product (89% based on 7-TES-baccatin III) with an effective HI of 99.3% (96.0% title product and 3.3% 7-TES-taxol).

Elemental Analysis (%) C ₅₇ H ₇₃ NO ₁₅ Si		
	Calcd.	Found
C	65.81	65.47
H	7.07	7.12
N	1.35	1.64

m.p. 153 - 155°C

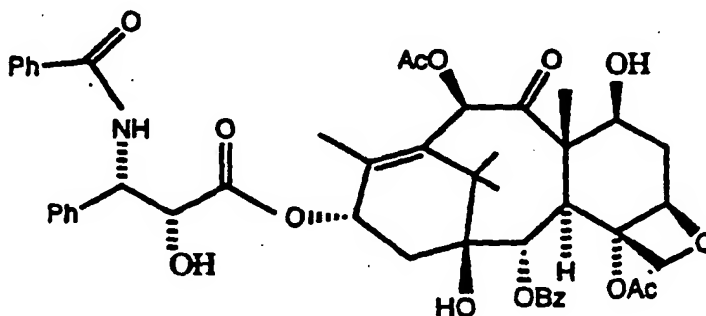
[α]_D: -59.6 (c 1, CHCl₃)

TLC: R_f = 0.31 Reverse Phase HPTLC, acetonitrile/water, 70:30, UV visualization.

Example 6

Preparation of Taxol [2aR-[2a α ,4 β ,4a β ,6 β ,9 α (α R*, β S*),11 α ,12 α ,12a α -12b α]]- β -(Benzoylamino)- α -hydroxy-benzenepropanoic acid, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4-11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]henz[1,2-b]oxet-9-yl-ester

[0099]



[0100] To a solution of the title product of Example 5 above ("Compound 5", 5.0 g, 4.81 mmol; HI 99.3% including Compound 5, HI 96.0 and 7-TES-taxol, HI 3.3) in ethanol (EtOH, 100 mL) and THF (80 mL) at 0 °C (Compound 5 was dissolved in EtOH/THF before cooling to 0 °C using an ice bath) was added precooled (~5 °C) 1.5 N HCl (aqueous, aq) dropwise with vigorous stirring over a period of 12 minutes. The cloudiness that appeared during the addition of 1.5 N HCl disappeared instantly. The resulting clear solution was stirred at 0 °C for 15 minutes and stored at 4 °C for 19.5 hrs. HPLC analysis of an aliquot (3 μ Phenyl BD column; 35% CH₃CN/65% H₂O linear gradient for 26 minutes; 100% CH₃CN linear gradient for 7 minutes; 35% CH₃CN/65% H₂O isocratic for 7 minutes) at this point indicated the presence of taxol (98.6%), 7-TES taxol (0.6%) and a polar impurity (0.3%) along with other minor impurities. The reaction mixture was diluted with ethyl acetate (EtOAc, 200 mL) and washed with cold (about 5 °C) NaHCO₃ (500 mL and 2 x 200 mL). Washing was continued until the pH of the aqueous washings was ~8.5.

[0101] The combined aqueous layer was extracted with EtOAc (2x100 mL). The organic layers were combined and washed with brine (300 mL), dried (Na₂SO₄, 100 g), filtered and concentrated to give crude taxol as a white solid (4.44 g; HPLC HI 97.7%). It was dissolved in 25 mL of methanol (MeOH)/isopropanol (IPA) (1:5.8) and diluted with H₂O (1.4 mL) by gentle warming (warmed to ~40 to 45 °C on a water bath). The resulting solution was stored in a hexane atmosphere (the container having the solution of crude taxol in MeOH/IPA/H₂O was placed in another larger container having hexane (20 mL) in a closed system at room temperature) at room temperature for 16 hrs. The white crystalline (visual examination under a microscope) solid was filtered, washed with cold (5 °C) hexane (25 mL) and dried under high vacuum to give 3.8 g (93.0%) of taxol with HPLC HI 99.0%. The mother liquor and the washings were concentrated under reduced pressure to give 0.28 g (7.0%) of a faint yellow solid (HPLC HI 80.6%) which was set aside for further processing at a later time.

Elemental Analysis (%) $C_{47}H_{51}NO_{14} \cdot 1.0 H_2O$		
	Calcd.	Found
C	64.74	64.71
H	6.13	6.48
N	1.61	1.57
KF(H ₂ O)	2.07	1.90

m.p. 211 - 213°C

$[\alpha]_D$: -51.5 (c 1, CHCl₃)

TLC: R_f = 0.22; MeOH:AcOEt:Hexane; 0.6:4.0:5.4; UV and PMA Visualization.

Example 7

Preparation of Taxol

[0102] To a 2 L polyethylene bottle containing a solution of Compound 5 (2'-MOP-7-triethylsilyltaxol, 20 g, 19.1 mmol) in acetonitrile (800 ml) and pyridine (48 ml) at 0°C was added dropwise 48% aqueous hydrofluoric acid (HF) (104 ml) over a 60 minute period. The internal temperature did not exceed 5°C during the addition. The clear solution was held at 4°C without agitation for a period of 24 hrs. The reaction was monitored by HPLC (Waters, Nova-Pak Phenyl, 3.9 x 150 mm column; absorption: 227 nm; flow rate: 2 ml/min) Chromatography condition:

0-26 min, 35% CH₃CN/65% H₂O to 100% CH₃CN, linear gradient, 26-28 min, 100% CH₃CN to 35% CH₃CN/65% H₂O, linear gradient, 28-35 min, 35% CH₃CN/65% H₂O, isocratic

Rt: 13.73 for 7-TES-taxol

Rt: 6.65 for Taxol

Rt: 4.96 for 10-desacetyl-taxol

[0103] After 19 hrs of reaction, 0.36% of 7-TES-taxol remained in this mixture. After 24 hrs, 7-TES-taxol and 10-desacetyl-taxol were not present (impurity index $II < 0.04\%$) in the reaction mixture. The solution was then diluted with ethyl acetate (1 L) and washed with 1N HCl (800 ml x 2). The combined aqueous layer was extracted with ethyl acetate (400 ml x 1). The organic layers were combined and washed with saturated aqueous sodium bicarbonate solution (800 ml x 5), brine (300 ml x 1), dried over sodium sulfate, filtered and concentrated to give 17.46 g (~100%) of crude taxol as a white solid. The HPLC HI for the crude taxol obtained above was 98.7%. The yield is uncorrected.

Elemental Analysis (%) $C_{47}H_{51}NO_{14} \cdot 1.3 H_2O$		
	Calcd.	Found
C	64.34	64.32
H	6.16	5.99
N	1.60	2.00
KF(H ₂ O)	2.67	2.00

m.p. 207.5 - 212°C (w/decomp)

$[\alpha]_D$: -52.5 (c 1, CHCl₃)

TLC: R_f = 0.22; Silica gel; MeOH:AcOEt:Hexane; 0.6:4.0:5.4; UV and PMA Visualization.

Example 8

Preparation of Taxol

[0104] To a solution of 2'-MOP-7-TES-taxol (Compound 5, 5.0 g, 4.81 mmol, HI 99.2% (including 2'-MOP-7-TES-taxol, HI 95.7) in ethanol (EtOH, 50 ml) and THF (40 ml) at 0°C (ice bath, 2'-MOP-7-TES-taxol was dissolved in EtOH/THF before cooling to 0°C) was added precooled (~5°C) 1.5 N HCl (aq., 50 ml) dropwise with vigorous stirring over a period of 40 minutes. The cloudiness that appeared during the addition of 1.5 N HCl disappeared instantly. The resulting clear solution was stirred at -2°C for 1 hour and stored at 4°C for 22 hours. A white solid about 100-200 mg (taxol) precipitated at this stage. (In process HPLC analysis of an aliquot after 20 hours (3μ Phenyl BD column); 35% CH₃CN/65% H₂O-linear gradient for 26 minutes; 100% CH₃CN-linear gradient for 7 minutes; 35% CH₃CN/65% H₂O-

isocratic for 7 minutes) at this point indicated the presence of taxol (97.2%), 7-TES taxol (0.2%), 10-desacetyl taxol (0.7%) with other minor impurities.) The reaction mixture was diluted with EtOAc (200 ml) and washed with cold ($\sim 5^{\circ}\text{C}$) NaHCO_3 (400 ml and 2 x 200 ml). (The pH of the aqueous washings should preferably be ~ 8.5 (where not, washing is preferably continued until the pH reaches 8.5)). The combined aqueous layer was extracted with EtOAc (2 x 80 ml). The organic layers were combined and washed with brine (200 ml), dried (Na_2SO_4 , 100 g), filtered and concentrated to give crude taxol as a white solid (4.2 g; HI 97.9%). It was dissolved in 31 ml of EtOH/heptane (6:4) and diluted with H_2O (0.15 ml) by gentle warming (warmed to $\sim 30\text{--}35^{\circ}\text{C}$ on a water bath). The resulting homogenous clear solution was stored at 4°C for about 20 hours. The white crystalline (visual examination under a microscope) solid was filtered, washed with cold (5°C) heptane (20 ml) and dried under high vacuum to give 3.72 g (90.6%) of taxol with HI 98.6%. The mother liquor and the washings on concentration under reduced pressure gave crude taxol (0.45 g) which on crystallization (dissolved in EtOH/heptane (0.5:0.3, 4.6 ml) and H_2O (20 μl) and stored at 4° for 20 hours) yielded the second crop of white crystalline (visual examination under a microscope) solid (0.18 g; 4.0%; HI 92.0%).

Elemental Analysis (%) $\text{C}_{47}\text{N}_{51}\text{NO}_{14} \cdot 2.55 \text{H}_2\text{O}$		
	Calc.	Found
C	62.73	62.35
H	6.28	6.43
N	1.56	1.94
H_2O	5.11	4.91

mp = $207 - 208^{\circ}\text{C}$

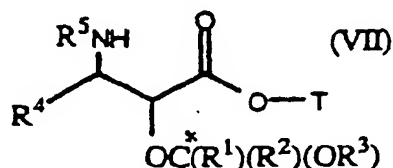
Opt. rot.: $[\alpha]_{\text{D}} = -52.3^{\circ}$ (c 1, CHCl_3)

TLC: $R_f = 0.22$; silica gel; MeOH:EtOAc:Hex, 0.6:4.0:5.4; UV and PMA visualization

HPLC: HI = 98.6%

Claims

1. A sidechain-bearing taxane of the following formula VII or a salt thereof:



where

R^1 and R^2 :

- (i) are both the same alkyl group;
- (ii) together with the carbon atom to which they are attached form a cycloalkyl group;
- (iii) together with the carbon atom to which they are attached form a cycloalkenyl group; or
- (iv) together with the carbon atom to which they are attached form a heterocyclo group;

wherein the carbon atom marked with an asterisk to which R^1 and R^2 are bonded is non-asymmetric;

R_3 is alkyl;

R_4 is aryl;

R^5 is hydrogen, arylcarbonyl, or alkyloxycarbonyl,

wherein

"alkyl" means such a group having 1-10 carbon atoms in a normal chain and being optionally substituted by

one or more of halo, alkoxy, alkylthio, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, hydroxy or protected hydroxy, carboxyl (-COOH), alkyloxycarbonyl, alkylcarbonyloxy, carbamoyl (NH₂-CO-), amino (-NH₂), mono- or dialkylamino, or thiol (-SH);

"cycloalkyl" means such a group having 1 to 3 rings and 3-7 carbon atoms per ring, the group being optionally substituted by "alkyl" as defined above or by one or more of the substituents included in the definition of "alkyl";

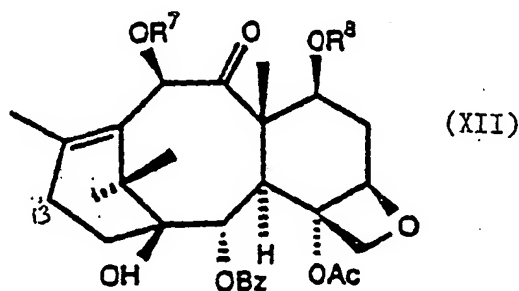
"cycloalkenyl" means "cycloalkyl" as defined above but containing at least one carbon-to-carbon double bond forming a partially unsaturated ring;

"aryl" means a homocyclic aromatic group containing 1 or 2 rings and 6 to 12 ring carbon atoms, and being optionally substituted by one or more nitro groups or as in the definition of "cycloalkyl" above;

"alkoxy" means "alkyl" as defined above but bonded through an oxygen linkage (-O-); and

"heterocyclo" means such a group having at least one hetero atom in at least one ring and optionally substituted as in the definition of "cycloalkyl" above,

and T is a taxane moiety bonded directly at C-13 of said moiety, the taxane moiety being of the formula:



where

Bz is benzoyl;

Ac is acetyl;

R⁷ is hydrogen, alkylcarbonyl or a hydroxyl protecting group; and

R⁸ is hydrogen or a hydroxyl protecting group,

2. A compound of claim 1, wherein R¹ and R² are both the same unsubstituted lower alkyl group, R³ is unsubstituted lower alkyl, R⁴ is phenyl, and R⁵ is benzoyl or t-butoxycarbonyl, "lower alkyl" meaning "alkyl" as defined in claim 1 but having 1 to 4 carbon atoms in the normal chain.
3. A compound according to claim 1 or claim 2 wherein R⁷ is acetyl.
4. A compound according to any one of claims 1 to 3 wherein R⁸ is H or trialkylsilyl.
5. A compound according to claim 1 which is 2'-MOP-7-triethylsilyl taxol.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 00 8433

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	EP 0 400 971 A (FLORIDA STATE UNIVERSITY) 5 December 1990 (1990-12-05) * claim 11; example 2 * * page 11, line 43 - page 12, line 10 * * page 12, line 38 - page 13, line 20 * ---	1-5	C07D305/14 A61K31/337 A61P35/00
P,X	WO 93 06079 A (FLORIDA STATE UNIVERSITY) 1 April 1993 (1993-04-01) * claims 1,6; example 20 * * page 13, line 20 - page 14, line 5 * ---	1-5	
P,X	WO 93 06094 A (FLORIDA STATE UNIVERSITY) 1 April 1993 (1993-04-01) * page 7, line 2 - page 8, line 6; claim 1; example 8 * * page 9, line 34 - page 10, line 16 * * page 15, line 8 - page 16, line 8 * ---	1-5	
Y	WO 90 10443 A (UNIVERSITY OF KANSAS) 20 September 1990 (1990-09-20) * claims 1,12 * -----	1-5	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C07D A61K
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 5 August 2003	Examiner Seymour, L
CATEGORY OF CITED DOCUMENTS X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document		T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons ----- &: member of the same patent family, corresponding document	

EPO FORM 1503 03.82 (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 00 8433

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-08-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0400971 A	05-12-1990	DD 341149 B1	07-05-1992
		US 5175315 A	29-12-1992
		AT 168997 T	15-08-1998
		AU 630696 B2	05-11-1992
		AU 5515090 A	20-12-1990
		BG 61074 B2	31-10-1996
		CA 2016951 A1	30-11-1990
		CA 2214051 A1	30-11-1990
		CN 1057049 A ,B	18-12-1991
		CN 1086512 A ,B	11-05-1994
		DE 69032512 D1	03-09-1998
		DE 69032512 T2	25-03-1999
		EG 19555 A	30-09-1995
		EP 0400971 A2	05-12-1990
		ES 2122957 T3	01-01-1999
		FI 102176 B1	30-10-1998
		HU 57199 A2	28-11-1991
		IE 62678 B1	22-02-1995
		IL 94426 A	15-06-1998
		JP 2111643 C	21-11-1996
		JP 3086860 A	11-04-1991
		JP 8030050 B	27-03-1996
		JP 2500198 B2	29-05-1996
		JP 7070057 A	14-03-1995
		KR 130463 B1	09-04-1998
		KR 130387 B1	09-04-1998
		MX 9203429 A1	01-07-1992
		NO 902404 A ,B,	03-12-1990
		NZ 233663 A	25-09-1992
		OA 9740 A	30-11-1993
		PL 285404 A1	11-02-1991
		PL 164539 B1	31-08-1994
		PT 94187 A ,B	08-02-1991
		RO 111766 B1	30-01-1997
		SG 43234 A1	17-10-1997
		RU 2097374 C1	27-11-1997
		US 5574156 A	12-11-1996
		US 5336785 A	09-08-1994
		YU 97590 A1	31-10-1991
		ZA 9003809 A	27-03-1991
		DK 400971 T3	26-04-1999
		US 2001037020 A1	01-11-2001
WO 9306079 A	01-04-1993	AT 146464 T	15-01-1997
		AT 128134 T	15-10-1995
		AT 231139 T	15-02-2003

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 00 8433

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-08-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9306079 A		AT 187170 T	15-12-1999
		AT 178060 T	15-04-1999
		AT 184004 T	15-09-1999
		AU 649875 B2	02-06-1994
		AU 2212292 A	25-03-1993
		AU 653247 B2	22-09-1994
		AU 2212392 A	25-03-1993
		AU 655493 B2	22-12-1994
		AU 2212492 A	25-03-1993
		AU 643911 B2	25-11-1993
		AU 2688892 A	27-04-1993
		AU 647971 B2	31-03-1994
		AU 2689092 A	27-04-1993
		AU 663732 B2	19-10-1995
		AU 2692692 A	27-04-1993
		AU 642391 B3	14-10-1993
		AU 3983793 A	19-08-1993
		AU 642392 B3	14-10-1993
		AU 3983893 A	19-08-1993
		CA 2077394 A1	24-03-1993
		CA 2077598 A1	24-03-1993
		CA 2077621 A1	24-03-1993
		CA 2098478 A1	24-03-1993
		CA 2098568 A1	24-03-1993
		CA 2119363 A1	01-04-1993
		CA 2221190 A1	24-03-1993
		CA 2254273 A1	24-03-1993
		CA 2418125 A1	24-03-1993
		CN 1075315 A ,B	18-08-1993
		CN 1075718 A ,B	01-09-1993
		CZ 9400660 A3	15-12-1994
		CZ 9400661 A3	12-07-1995
		CZ 9400662 A3	15-12-1994
		CZ 289299 B6	12-12-2001
		DE 69204951 D1	26-10-1995
		DE 69216028 D1	30-01-1997
		DE 69228756 D1	29-04-1999
		DE 69228756 T2	07-10-1999
		DE 69229916 D1	07-10-1999
		DE 69229916 T2	17-02-2000
		DE 69230379 D1	05-01-2000
		DE 69230379 T2	25-05-2000
		DE 69232895 D1	20-02-2003
		DE 69232895 T2	31-07-2003
		DK 534707 T3	09-06-1997
		DK 534708 T3	05-02-1996

EPO FORM P4559

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO.

EP 03 00 8433

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-08-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9306079 A		DK 534709 T3	12-05-2003
WO 9306094 A	01-04-1993	US 5229526 A	20-07-1993
		AT 146464 T	15-01-1997
		AT 128134 T	15-10-1995
		AT 231139 T	15-02-2003
		AT 187170 T	15-12-1999
		AT 178060 T	15-04-1999
		AT 184004 T	15-09-1999
		AU 649875 B2	02-06-1994
		AU 2212292 A	25-03-1993
		AU 653247 B2	22-09-1994
		AU 2212392 A	25-03-1993
		AU 655493 B2	22-12-1994
		AU 2212492 A	25-03-1993
		AU 643911 B2	25-11-1993
		AU 2688892 A	27-04-1993
		AU 647971 B2	31-03-1994
		AU 2689092 A	27-04-1993
		AU 663732 B2	19-10-1995
		AU 2692692 A	27-04-1993
		AU 642391 B3	14-10-1993
		AU 3983793 A	19-08-1993
		AU 642392 B3	14-10-1993
		AU 3983893 A	19-08-1993
		CA 2077394 A1	24-03-1993
		CA 2077598 A1	24-03-1993
		CA 2077621 A1	24-03-1993
		CA 2098478 A1	24-03-1993
		CA 2098568 A1	24-03-1993
		CA 2119363 A1	01-04-1993
		CA 2221190 A1	24-03-1993
		CA 2254273 A1	24-03-1993
		CA 2418125 A1	24-03-1993
		CN 1075315 A ,B	18-08-1993
		CN 1075718 A ,B	01-09-1993
		CZ 9400660 A3	15-12-1994
		CZ 9400661 A3	12-07-1995
		CZ 9400662 A3	15-12-1994
		CZ 289299 B6	12-12-2001
		DE 69204951 D1	26-10-1995
		DE 69216028 D1	30-01-1997
		DE 69228756 D1	29-04-1999
		DE 69228756 T2	07-10-1999
		DE 69229916 D1	07-10-1999
		DE 69229916 T2	17-02-2000

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 00 8433

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-08-2003

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9306094	A		DE 69230379 D1	05-01-2000
			DE 69230379 T2	25-05-2000
			DE 69232895 D1	20-02-2003
			DE 69232895 T2	31-07-2003
			DK 534707 T3	09-06-1997
			DK 534708 T3	05-02-1996

WO 9010443	A	20-09-1990	US 4960790 A	02-10-1990
			AU 628161 B2	10-09-1992
			AU 5271590 A	09-10-1990
			CA 2028096 A1	10-09-1990
			CN 1058018 A	22-01-1992
			DD 296485 A5	05-12-1991
			EP 0419653 A1	03-04-1991
			GR 90100523 A ,B	10-12-1991
			HU 56270 A2	28-08-1991
			JP 4504845 T	27-08-1992
			OA 9458 A	15-11-1992
			WO 9010443 A1	20-09-1990
			ZA 9005229 A	29-05-1991

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82